



**DCode™ Control Reagent Kit for
SSCP**

Instruction Manual

**Catalog Number
170-9151**

BIO-RAD

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* The Polymerase Chain Reaction (PCR) process is covered by patents owned by Hoffman-La Roche.

Section 1 Introduction

The DCode control kit for SSCP (Single Stranded Conformational Polymorphism) provides reagents that are used to prepare mutant and wild-type DNA for gel electrophoresis with the DCode universal mutation detection system. The SSCP technique is based on the fact that single-stranded DNA has a defined secondary structure. Sequence differences as small as a single base change can affect this secondary structure and can be detected by electrophoresis in a non-denaturing polyacrylamide gel.¹ Double stranded mutant and wild-type samples are first denatured into single strands and then loaded onto the gel. Differences in mobility of the single strands between the wild-type DNA and the other samples indicate a mutation. SSCP is a widely used mutation screening method because of its simplicity.

The DCode control kit for SSCP contains wild-type DNA, mutant DNA, two 19 base-pair oligonucleotide primers, and 2x SSCP gel loading dye. The mutant DNA is identical to the wild-type DNA, except for a single A to T mutation at fragment base number 110. The PCR reaction will produce a 299 bp fragment. There are sufficient amounts of DNA and primers for five 100 µl PCR reactions for both the mutant and wild-type DNA.

1.1 Kit Components

Item	Concentration	Amount	Volume
Mutant DNA	50 pg/µl	500 pg	10 µl
Wild-type DNA	50 pg/µl	500 pg	10 µl
Primer A	10 pmol/µl	400 pmol	40 µl
Primer B	10 pmol/µl	400 pmol	40 µl
2x SSCP Gel Loading Dye	2x		1 ml

Sequence of Primer A: 5'- GGG CTG GGC ATA AAA GTC A -3'
 Sequence of Primer B: 5'- AAT AGA CCA ATA GGC AGA G -3'
 2x SSCP Gel Loading Dye: 95% Formamide, 20 mM EDTA pH 8.0,
 0.05% Xylene Cyanol, and 0.05%
 Bromophenol Blue

1.2 Additional Supplies Required

Taq DNA polymerase enzyme
 10x *Taq* polymerase buffer
 2.5 mM dNTPs
 Sterile water
 Thin-walled microfuge tubes - 200 µl or 500 µl size
 Sterile aerosol tips

1.3 Storage Conditions

All kit components should be stored at -20 °C. The shelf life of the kit stored at -20 °C is 1 year.

Section 2 PCR Reaction

Note: It is extremely important that solutions and materials used during PCR set-up are not exposed to amplified DNA to avoid contamination during amplification.

1. Add the components listed below to 200 µl or 500 µl thin-walled microfuge tubes and mix.

	Tube 1 Wild-type DNA	Tube 2 Mutant DNA
Control DNA	1.5 µl	1.5 µl
Primer A	2.5 µl	2.5 µl
Primer B	2.5 µl	2.5 µl
10x <i>Taq</i> polymerase buffer	10.0 µl	10.0 µl
2.5 mM dNTPs	8.0 µl	8.0 µl
<i>Taq</i> DNA polymerase enzyme (5 U/µl)	0.5 µl	0.5 µl
Sterile water	75.0 µl	75.0 µl
Total volume	100.0 µl	100.0 µl

Note: 10x *Taq* polymerase buffer contains 100 mM Tris-HCl pH 8.3, 500 mM KCl, 15 mM MgCl₂, and 0.01% gelatin. If desired, add a third tube which contains no control DNA as a negative control.

2. Place the tubes into a thermocycler and enter the following parameters.

Step 1	94 °C	2 minutes	x 1 cycle
Step 2	94 °C	45 seconds	x 35 cycles
	61 °C	45 seconds	
	72 °C	45 seconds	
Step 3	72 °C	10 minutes	x 1 cycle
3. To check the product, add 5 µl of the amplified DNA to gel loading buffer and run on a 10% acrylamide/bis gel (10% Ready Gel—catalog number 161-0905). Run a DNA size standard, such as Bio-Rad's

100 bp Molecular Ruler (catalog number 170-8202), on the gel to approximate the size of the product. The size of the product should be 299 base pairs in length.

4. Store the amplified DNA at 4 °C. For long term storage (> 2 weeks), store the amplified DNA at -20 °C.

Section 3 SSCP Gel

In SSCP, a nondenaturing polyacrylamide gel is used to separate single-stranded DNA. Altered conformation due to a mutation in the sequence can cause the mutant single-stranded DNA to migrate differently than the wild-type DNA. This migration difference can be seen as a band shift between the mutant and wild-type DNA samples.

Note: Refer to the DCode universal mutation detection system manual for information on correct casting and running an SSCP gel on the DCode Universal Mutation Detection system.

1. Add 5 µl of the amplified mutant DNA and 5 µl of 2x SSCP gel loading dye to a microfuge tube. Gently mix the contents of the tube. Do the same for the amplified wild-type DNA.
2. Place the tubes (mutant and wild-type) into a 95 °C water bath for 7 minutes and then on ice for about 5 minutes.
3. Load 10 µl of the samples into the wells of an 8% acrylamide/bis gel (37.5:1), containing 7% glycerol, and 1x TBE buffer.

Note: Recipe for a 20 x 20 x 0.1 cm gel format.

40% Acrylamide/bis (37.5:1)	8 ml
10x TBE	4 ml
100% Glycerol	2.8 ml
TEMED	40 µl
10% Ammonium persulfate	400 µl
dH ₂ O	24.8 ml

4. Run the gel under the conditions listed below.

Buffer	1x TBE
Constant power	30 W
Buffer temperature	10 °C
Run time	3.5 hours

Note: Pre-chill the running buffer prior to a run.

5. After the run is completed, the gel can be stained with ethidium bromide (1 µg/ml) in 1x TBE buffer solution for about 5 minutes or SyBr Green II (Molecular Probes—1:10,000 dilution) in 1x TBE buffer solution for about 30–45 minutes. Visualize and photograph the gel by placing it on a UV transilluminator (Gel Documentation System 1000, catalog numbers 170-7520 through 170-7527, or Bio-Rad Polaroid Gel Documentation System, catalog numbers 170-3742 through 170-3749).
6. The result from your gel should look similar to that in Figure 1. There should be a band shift between the single-strands of the mutant and wild-type fragments.

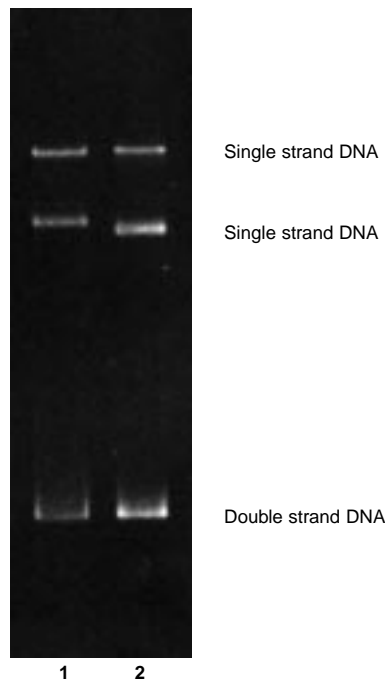


Fig. 1. Separation of mutant and wild-type DNA on the DCode system. The samples were electrophoresed on an 8% acrylamide/bis gel containing 7% glycerol for 3.5 hours at 30 W with the buffer chilled at 10 °C. The gel was stained with SyBr Green II. Lane 1 contains the mutant DNA and lane 2 contains the wild-type DNA.

Section 4 Troubleshooting

Refer to the DCode universal mutation detection system manual for more troubleshooting details.

4.1 PCR

Problem	Cause	Solution
Faint, visible bands	Unused primers	1. A 19 bp fragment is not a problem.
	Concatemer DNA	2. Amplified DNA forms concatemer size DNA. This is not a problem when the sample is denatured for the SSCP gel.
No 299 bp fragment	No DNA	1. Make sure DNA is added to PCR reaction.
	Inactive enzyme	2. Use viable <i>Taq</i> DNA polymerase.
	Missing or bad dNTPs	3. Make sure all four dNTPs are used in PCR reaction.
	Missing primer/primers	4. Make sure both primers are used in PCR reaction.
Numerous bands	Nonspecific priming	1. Do set-up in PCR designated area.
		2. Make sure not to cross contaminate the mutant and wild-type DNA.

4.2 SSCP Gel

Problem	Cause	Solution
Streaking or DNA spikes in gel	Impurities in acrylamide	<ol style="list-style-type: none">1. Filter before use. Check shelf life date of acrylamide solution.2. Be careful not to pierce wells while loading samples.
No difference in band shift	Incorrect running conditions.	<ol style="list-style-type: none">1. Insure that the gel contains 7% glycerol.2. Run the gel at 10 °C.3. Denature samples prior to loading.

Section 5 References

1. Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K. and Sekiya, T, *Proc. Natl. Acad. Sci. USA*, **86**, 2766-2770 (1989).